

Claims

What is claimed is:

1. A method for delivering a compound into a cell comprising administering to the cell a composition which comprises the compound to be delivered
5 and an organic halide.
2. A method of claim 1 wherein said composition further comprises a carrier.
3. A method of claim 2 wherein said organic halide is selected from the group consisting of a gaseous organic halide and a liquid organic halide.
- 10 4. A method of claim 2 wherein said organic halide is a gas.
5. A method of claim 2 wherein said organic halide is a liquid.
6. A method of claim 2 wherein said organic halide is a gaseous precursor.
7. A method of claim 2 wherein said organic halide is a fluorinated
15 compound.
8. A method of claim 2 wherein said organic halide is a perfluorinated compound.
9. A method of claim 2 wherein said organic halide is a perfluorocarbon.
- 20 10. A method of claim 2 wherein said organic halide is a perfluoroether compound.

11. A method of claim 9 wherein said perfluorocarbon is a liquid.
12. A method of claim 11 wherein said liquid perfluorocarbon is a gaseous precursor.
13. A method of claim 9 wherein said perfluorocarbon is a gas.
- 5 14. A method of claim 2 wherein said organic halide is selected from the group consisting of 1-bromo-nonafluorobutane, perfluorooctyl iodide, perfluorooctyl bromide, 1-chloro-1-fluoro-1-bromomethane, 1,1,1-trichloro-2,2,2-trifluoroethane, 1,2-dichloro-2,2-difluoroethane, 1,1-dichloro-1,2-difluoroethane, 1,2-dichloro-1,1,3-trifluoropropane, 10 1-bromoperfluorobutane, 1-bromo-2,4-difluorobenzene, 2-iodo-1,1,1-trifluoroethane, 5-bromovaleryl chloride, 1,3-dichlorotetrafluoroacetone, bromine pentafluoride, 1-bromo-1,1,2,3,3,3-hexafluoropropane, 2-chloro 1,1,1,4,4,4-hexafluoro-2-butene, 2-chloropentafluoro-1,3-butadiene, iodotrifluoroethylene, 1,1,2-trifluoro-2-chloroethane, 1,2-difluorochloroethane, 1,1-difluoro-2-chloroethane, 15 1,1-dichlorofluoroethane, heptafluoro-2-iodopropane, bromotrifluoroethane, chlorotrifluoromethane, dichlorodifluoromethane, dibromofluoromethane, chloropentafluoroethane, bromochlorodifluoromethane, dichloro-1,1,2,2-tetrafluoroethane, 1,1,1,3,3-pentafluoropentane, perfluorotributylamine, perfluorotripropylamine, 3-fluorobenzaldehyde, 2-fluoro-5-nitrotoluene, 20 3-fluorostyrene, 3,5-difluoroaniline, 2,2,2-trifluoroethylacrylate, 3-(trifluoromethoxy)-acetophenone, 1,1,2,2,3,3,4,4-octafluorobutane, 1,1,1,3,3-pentafluorobutane, 1-fluorobutane, 1,1,2,2,3,3,4,4-octafluorobutane, 1,1,1,3,3-pentafluorobutane, perfluoro-4 methylquinolizidine, perfluoro-N-methyl-decahydroquinone, perfluoro-N-methyl-decahydroisoquinone, perfluoro-N-cyclohexyl- 25 pyrrolidine, tetradecaperfluoroheptane, dodecaperfluorocyclohexane, perfluoromethane, perfluoroethane, perfluoropropane, perfluorobutane, perfluoropentane, perfluorohexane, perfluoroheptane, perfluorooctane, perfluorononane, perfluorodecane, perfluorododecane, perfluoro-2-methyl-2-pentene, perfluorocyclohexane, perfluorodecalin, perfluorododecalin, perfluoropropylene, perfluorocyclobutane,

perfluoro-2-butyne, perfluoro-2-butene, perfluorobuta-1,3-diene, perfluorobutylethyl ether, bis(perfluoroisopropyl) ether, bis(perfluoropropyl) ether, perfluorotetrahydropyran, perfluoromethyl tetrahydrofuran, perfluoro t-butyl methyl ether, perfluoro isobutyl methyl ether, perfluoro n-butyl methyl ether, perfluoro isopropyl ethyl ether, perfluoro n-propyl ethyl ether, perfluoro cyclobutyl methyl ether, perfluoro cyclopropyl ethyl ether, perfluoro isopropyl methyl ether, perfluoro n-propyl methyl ether, perfluoro diethyl ether, perfluoro cyclopropyl methyl ether, perfluoro methyl ethyl ether, perfluoro dimethyl ether, sulfur hexafluoride, and selenium hexafluoride.

10 15. A method of claim 2 wherein said organic halide is selected from the group consisting of perfluoromethane, perfluoroethane, perfluoropropane, perfluorobutane, perfluoropentane, perfluorohexane, perfluoroheptane, perfluorooctane, perfluorononane, perfluorodecane, perfluorododecane, perfluoro-2-methyl-2-pentene, perfluorocyclohexane, perfluorodecalin, perfluorododecalin, tetradecaperfluorohexane, and dodecaperfluorocyclohexane.

 16. A method of claim 2 wherein said organic halide is selected from the group consisting of perfluorobutylethyl ether, bis(perfluoroisopropyl) ether, bis(perfluoropropyl) ether, perfluorotetrahydropyran, perfluoromethyl tetrahydrofuran, perfluoro t-butyl methyl ether, perfluoro isobutyl methyl ether, perfluoro n-butyl methyl ether, perfluoro isopropyl ethyl ether, perfluoro n-propyl ethyl ether, perfluoro cyclobutyl methyl ether, perfluoro cyclopropyl ethyl ether, perfluoro isopropyl methyl ether, perfluoro n-propyl methyl ether, perfluoro diethyl ether, perfluoro cyclopropyl methyl ether, perfluoro methyl ethyl ether, and perfluoro dimethyl ether.

 17. A method of claim 2 wherein said organic halide is selected from the group consisting of perfluorohexane and perfluorocyclohexane.

 18. A method of claim 2 further comprising applying ultrasound to said cell.

19. A method of claim 18 wherein said ultrasound is applied at a frequency from about 40 kilohertz to about 25 megahertz, and an energy level of from about 500 milliwatts/cm² to about 10 watts/cm².

20. A method of claim 18 wherein said ultrasound is applied at a
5 frequency of from about 500 kilohertz to about 200 kilohertz and said energy level is from about 500 milliwatts/cm² to about 200 milliwatts/cm².

21. A method of claim 18 wherein said ultrasound is applied at a frequency of from about 20 megahertz to about 1 megahertz and said energy level is from about 200 milliwatts/cm² to about 100 milliwatts/cm².

10 22. A method of claim 21 wherein said ultrasound is applied at a duty cycle of from about 1% to about 100% of the treatment time.

23. A method of claim 18 wherein said compound and said ultrasound are administered and applied simultaneously.

24. A method of claim 2 wherein said carrier is selected from the group
15 consisting of polymers, lipids, proteins, and metal ions.

25. A method of claim 24 wherein said carrier is a protein.

26. A method of claim 25 wherein said protein is a cationic protein.

27. A method of claim 26 wherein said cationic protein is selected from the group consisting of polylysine and polyethyleneimine.

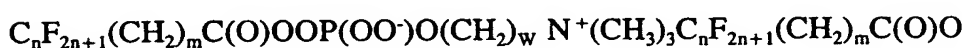
20 28. A method of claim 24 wherein said carrier is a lipid.

29. A method of claim 28 wherein said lipid is a cationic lipid.

30. A method of claim 29 wherein the carrier is a cationic lipid and said cationic lipid is N-[1(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride.

31. A method of claim 28 wherein said lipid is a fluorinated lipid.

32. A method of claim 31 wherein said fluorinated lipid is selected
5 from compounds of the formula



wherein: m is 0 to about 18, n is 1 to about 12; and w is 1 to about 8.

33. A method of claim 28 wherein said lipid is a fluorinated
phospholipid.

10 34. A method of claim 24 wherein said carrier is a polymer.

35. A method of claim 34 wherein said polymer is selected from the group consisting of polyethylenes, polyoxyethylenes, polypropylenes, pluronic acids and alcohols, polyvinyls, polyvinylpyrrolidone, arabinans, fructans, fucans, galactans, galacturonans, glucans, mannans, xylans, levan, fucoidan, carrageenan,
15 galactocarolose, pectin, pectic acid, amylose, pullulan, glycogen, amylopectin, cellulose, carboxymethylcellulose, hydroxypropyl methylcellulose, dextran, pustulan, chitin, agarose, keratan, chondroitin, dermatan, hyaluronic acid, alginic acid, homopolymers and heteropolymers containing one or more of an aldose, ketose, acid, amine, erythrose, threose, ribose, arabinose, xylose, lyxose, allose, altrose, glucose,
20 mannose, gulose, idose, galactose, talose, erythrulose, ribulose, xylulose, psicose, fructose, sorbose, tagatose, glucuronic acid, gluconic acid, glucaric acid, galacturonic acid, mannuronic acid, guluronic acid, glucosamine, galactosamine and neuraminic acid.

36. A method of claim 24 wherein said carrier is a metal ion.

37. A method of claim 24 wherein said carrier is a metal ion and said metal ion is selected from the group consisting of calcium ions, magnesium ions, and zinc ions.

38. A method of claim 2 wherein said carrier is selected from the group
5 consisting of Lipofectin, Lipofectamine, Transfectace, Transfectam, Cytofectin, DMRIE, DLRIE, GAP-DLRIE, DOTAP, DOPE, DMEAP, DODMP, DOPC, DDAB, DOSPA, EDLPC, EDMPC, DPH, TMADPH, CTAB, lysyl-PE, DC-Chol, -alanyl cholesterol, DCGS, DPPES, DCPE, DMAP, DMPE, DOGS, DOHME, DPEPC, Pluronic, Tween, BRIJ, plasmalogen, phosphatidylethanolamine, phosphatidylcholine,
10 glycerol-3-ethylphosphatidylcholine, dimethyl ammonium propane, trimethyl ammonium propane, diethylammonium propane, triethylammonium propane, dimethyldioctadecylammonium bromide, a sphingolipid, sphingomyelin, a lysolipid, a glycolipid, a sulfatide, a glycosphingolipid, cholesterol, cholesterol ester, cholesterol salt, oil, N-succinyldioleoylphosphatidylethanolamine, 1,2,-dioleoyl-sn-glycerol,
15 1,3-dipalmitoyl-2-succinylglycerol, 1,2-dipalmitoyl-sn-3-succinylglycerol, 1-hexadecyl-2-palmitoylglycerophosphatidylethanolamine, palmitoylhomocystiene, N,N'-Bis (dodecylaminocarbonylmethylene)-N,N'-bis ((-N,N,N-trimethylammoniummethyl-aminocarbonylmethylene)ethylenediamine tetraiodide; N,N''-Bis (hexadecylaminocarbonylmethylene)-N,N',N''-tris
20 ((-N,N,N-trimethylammonium-ethylaminocarbonylmethylenediethylenetriamine hexaiodide; N,N'-Bis (dodecylaminocarbonylmethylene)-N,N''-bis((-N,N,N-trimethylammoniummethylaminocarbonylmethylene)cyclohexylene-1,4-diamine tetraiodide; 1,1,7,7-tetra-((-N,N,N,N-tetramethylammoniummethylamino-carbonylmethylene)-3-hexadecylaminocarbonyl-methylene-1,3,7-triazaheptane
25 heptaoidide; and N,N,N',N'-tetra((-N,N,N-trimethylammonium-ethylaminocarbonylmethylene)-N'-(1,2-dioleoylglycero-3-phosphoethanolaminocarbonylmethylene) diethylenetriamine tetraiodide.

39. A method of claim 2 wherein said carrier is selected from the group consisting of dioleoylphosphatidylethanolamine, a fatty acid, a lysolipid,
30 phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine,

phosphatidylglycerol, phosphatidylinositol, a sphingolipid, a glycolipid, a glucolipid, a sulfatide, a glycosphingolipid, phosphatidic acid, palmitic acid, stearic acid, arachidonic acid, oleic acid, a lipid bearing a polymer, a lipid bearing a sulfonated saccharide, cholesterol, tocopherol hemisuccinate, a lipid with an ether-linked fatty acid, a lipid with an ester-linked fatty acid, a polymerized lipid, diacetyl phosphate, 5 stearylamine, cardiolipin, a phospholipid with a fatty acid of 6-8 carbons in length, a phospholipid with asymmetric acyl chains, 6-(5-cholesten-3 β -yloxy)-1-thio-b-D-galactopyranoside, digalactosyldiglyceride, 6-(5-cholesten-3 β -yloxy)hexyl-6-amino-6-deoxy-1-thio-b-D-galactopyranoside, 6-(5-cholesten-3 β -yloxy)hexyl-6-amino-6-deoxyl-1-thio- α -D-mannopyranoside, 12-(((7'-diethylamino-coumarin-3-yl)carbonyl)methylamino)-octadecanoic acid; N-[12-(((7'-diethylaminocoumarin-3-yl)carbonyl)methyl-amino) octadecanoyl]-2-aminopalmitic acid; cholesteryl)4'-trimethyl-ammonio)butanoate; N-succinyldioleoyl-phosphatidylethanolamine; 1,2-dioleoyl-sn-glycerol; 1,2-dipalmitoyl-sn-3-succinyl-glycerol; 15 1,3-dipalmitoyl-2-succinylglycerol; 1-hexadecyl-2-palmitoylglycero-phosphoethanolamine, palmitoylhomocysteine, and/or combinations thereof.

40. A method of claim 39 wherein said carrier comprises phosphatidylcholine and said phosphatidylcholine is selected from the group consisting of dioleoylphosphatidylcholine, dimyristoylphosphatidylcholine, 20 dipentadecanoylphosphatidylcholine, dilauroylphosphatidylcholine, dioleoylphosphatidylcholine, dipalmitoylphosphatidylcholine, and distearoylphosphatidylcholine.

41. A method of claim 39 wherein said carrier comprises phosphatidylethanolamine and said phosphatidylethanolamine is 25 dioleoylphosphatidylethanolamine.

42. A method of claim 39 wherein said carrier comprises sphingolipid and said sphingolipid is sphingomyelin.

43. A method of claim 39 wherein said carrier comprises a glycolipid and said glycolipid is selected from ganglioside GM1 and ganglioside GM2.

44. A method of claim 39 wherein said carrier comprises a lipid bearing polymer and said polymer of said lipid bearing a polymer is selected from the
5 group consisting of polyethylene glycol, chitin, hyaluronic acid, and polyvinylpyrrolidone.

45. A method of claim 44 wherein said polymer is polyethylethylene glycol and said polyethylene glycol is selected from the group consisting of polyethylene glycol having a molecular weight of about 2000, 5000, and 8000.

10 46. A method of claim 39 wherein said carrier comprises a lipid bearing a sulfonated saccharide.

47. A method of claim 39 wherein said carrier comprises cholesterol and said cholesterol is selected from the group consisting of cholesterol sulfate and cholesterol hemisuccinate.

15 48. A method of claim 39 wherein said carrier comprises a phospholipid with asymmetric acyl chains and said phospholipid with asymmetric acyl chains is a phospholipid having one acyl chain of about 6 carbons in length and another acyl chain of about 12 carbons in length.

49. A method of claim 2 wherein said carrier comprises about 82 mole
20 percent dipalmitoylphosphatidylcholine, about 8 mole percent dipalmitoylphosphatidylethanolamine-polyethyleneglycol 5000 and about 10 mole percent dipalmitoylphosphatidic acid.

50. A method of claim 2 wherein said carrier is a surfactant.

51. A method of claim 50 where said surfactant is a fluorosurfactant.

52. A method of claim 2 wherein said organic halide is a gaseous precursor and wherein said gaseous precursor is converted to a gas after administration.

53. A method of claim 52 wherein said gaseous precursor is converted
5 to a gas by applying heat to said cell.

54. A method of claim 53 wherein said heat is applied by ultrasound.

55. A method of claim 54 wherein said ultrasound is applied at a frequency from about 40 kilohertz to about 25 megahertz, and said energy level is from about 500 milliwatts/cm² to about 10 watts/cm².

10 56. A method of claim 54 wherein said ultrasound is applied at a frequency of from about 500 kilohertz to about 200 kilohertz and said energy level is from about 500 milliwatts/cm² to about 200 milliwatts/cm².

57. A method of claim 54 wherein said ultrasound is applied at a frequency of from about 20 megahertz to about 1 megahertz and said energy level is
15 from about 200 milliwatts/cm² to about 100 milliwatts/cm².

58. A method of claim 54 wherein said ultrasound is applied at a duty cycle of from about 1% to about 100% treatment time.

59. A method of claim 54 wherein said ultrasound is applied at a duty cycle of from about 10% to about 20%.

20 60. A method of claim 2 wherein said method is carried out *in vivo*.

61. A method of claim 2 wherein said method is carried out *ex vivo*.

62. A method of claim 2 wherein said method is carried out *in vitro*.

63. A method of claim 2 wherein said carrier comprises a vesicle.

64. A method of claim 63 wherein said compound to be delivered is encapsulated in said vesicle.

65. A method of claim 2 wherein said cell is a mammalian cell.

5 66. A method of claim 65 wherein said mammalian cell is a human cell.

67. A method of claim 2 wherein said compound to be delivered is a nucleotide sequence.

10 68. A method of claim 67 wherein said nucleotide sequence is a DNA sequence.

69. A method of claim 68 wherein said DNA sequence is selected from the group consisting of a DNA sequence coding for interleukin 2, interleukin 4, Bcl 2, vascular endothelial growth factor, human papilloma virus, and human immunodeficiency virus, human leukocyte antigen, tumor necrosis factor, granulocyte-
15 macrophage colony stimulating factor, cystic fibrosis transmembrane receptor, adenosine deaminase, luteinizing hormone releasing hormone, luteinizing hormone releasing hormone antagonist, human growth hormone, insulin, high density lipoprotein, thymidine kinase, HLA-B7, Factor VIII, and *ras/p53*.

20 70. A method of claim 68 wherein said DNA sequence is administered for the purpose of treating a disease selected from the group consisting of acquired immune deficiency syndrome, hemophilia, muscular dystrophy, cystic fibrosis, diabetes, atherosclerosis, liver cancer, lung cancer, prostate cancer, ovarian cancer, brain cancer, kidney cancer melanoma, neuroblastoma, and breast cancer.

71. A method of claim 68 wherein said DNA sequence is administered by an inhaler for the purpose of treating cystic fibrosis.

72. A method of claim 68 wherein said DNA sequence is administered upon a balloon catheter for the purpose of treating atherosclerosis.

5 73. A method of claim 68 wherein said DNA sequence codes for vascular endothelial growth factor and said DNA sequence is administered in combination with a carrier wherein said carrier is a hydrogel.

10 74. A method of claim 68 wherein said DNA sequence codes for interleukin-2 and said DNA sequence is administered in combination with a cationic liposome.

75. A method of claim 68 wherein said DNA sequence is administered for the purpose of treating muscular dystrophy.

15 76. A method of claim 68 wherein said DNA sequence codes for Bcl 2 and said DNA sequence is administered for the purpose of treating colon cancer.

77. A method of claim 2 wherein the compound to be delivered is a bioactive agent.

78. A method of claim 2 wherein the compound to be delivered is a diagnostic agent.

20 79. A method of claim 2 wherein the compound to be delivered is a pharmaceutical agent.

80. A method of claim 2 wherein said method is carried out concurrently with another intracellular delivery technique selected from the group

consisting of calcium phosphate precipitation, transfection with a viral vector, microinjection and electroporation.

81. A method of claim 18 wherein said method is carried out concurrently with another intracellular delivery technique selected from the group
5 consisting of calcium phosphate precipitation, transfection with a viral vector, microinjection and electroporation.

82. A method of effecting the expression of a nucleotide sequence in a cell comprising administering to said cell a composition comprising a nucleotide sequence and an organic halide.

10 83. A method of claim 82 wherein said composition further comprises a carrier.

84. A method of claim 83 further comprising the step of applying ultrasound.

85. A method of claim 83 wherein said cell is a mammalian cell.

15 86. A method of claim 83 wherein said cell is a human cell.

87. A method of treating a patient comprising administering to the patient a composition comprising a therapeutically effective amount of a compound and an organic halide.

20 88. A method according to claim 87 further comprising the step of applying ultrasound.

89. A composition comprising a therapeutically effective amount of a compound and an organic halide.

90. A composition of claim 89 further comprising a carrier.

91. A composition comprising a diagnostically effective amount of a compound and an organic halide.

92. A composition of claim 91 further comprising a carrier.

5 93. A kit comprising an organic halide and a carrier.

94. A kit of claim 93 further comprising a compound to be delivered to a cell.

95. A kit of claim 93 further comprising conventional kit components.

10 96. A kit comprising an organic halide and a compound to be delivered to a cell.

97. A kit of claim 96 further comprising a carrier.

98. A kit of claim 96 further comprising conventional kit components.

99. A method for delivering a compound into a cell comprising administering to the cell the compound to be delivered, and applying ultrasound.

15 100. A method of claim 99 wherein said compound is in a composition which further comprises a carrier.

101. A method of treating a patient comprising administering to a patient a therapeutically effective amount of a compound, and applying ultrasound.

20 102. A method of claim 101 wherein said compound is in a composition which further comprises a carrier.

103. A method of effecting the expression of a nucleotide sequence in a cell comprising administering to the cell a nucleotide sequence, and applying ultrasound.

104. A method of claim 103 wherein said nucleotide sequence is in a
5 composition which further comprises a carrier.